PARASITOLOGY

Fishing for Answers to Whirling Disease

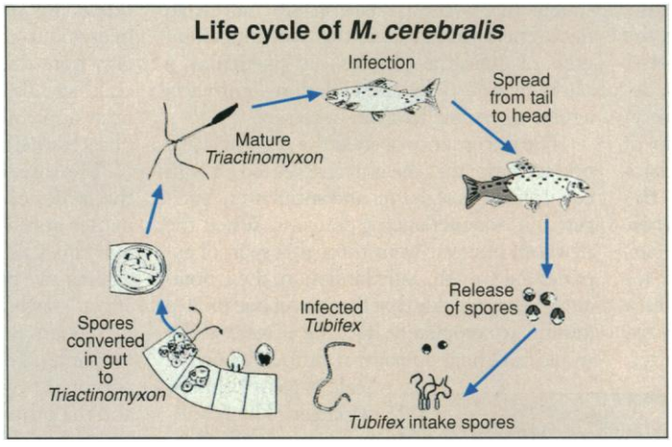
BOZEMAN, MONTANA—When virologist Karl Johnson retired from a storied career at the Centers for Disease Control and Prevention 10 years ago, he moved here to indulge a lifelong passion. Instead of battling Bolivian hemorrhagic fever, Ebola, and other devastating diseases, Johnson envisioned tooling away much of his time by casting his line into the Madison River, famous for its wild rainbow trout. But Johnson, instead, finds himself battling yet another pathogen—except this one targets not humans, but the trout. "Vandals have broken into the cathedral of fly-fishing," laments Johnson.

The "vandals" he's referring to are the protozoa *Myxobolus cerebralis*, parasites that infect young fish, devouring their cartilage and leaving them deformed. Inflammation meant to fight the infection puts pressure on nerves and disrupts balance, causing fish to swim in circles, or whirl, which makes it difficult to feed and escape predators. Because of "whirling disease," the rainbow trout population on the Madison has plummeted from 3500 fish per mile in 1990 to 300 per mile in 1994. Johnson and others responded by establishing the privately funded Whirling Disease Foundation in Bozeman, where he serves as scientific director. With money from the foundation, the federal government, and other resources, researchers are beginning to make dramatic progress against this puzzling disease.

First identified in Europe in 1893, but not seen in Montana until 3 years ago, whirling disease has been notoriously difficult to diagnose in early stages of infection. But a sensitive test, developed in the past year, has given researchers a new tool to track infections and study the complex life cycle of *M. cerebralis*, which depends on two hosts and two pathogenic stages. These new techniques should immediately help fisheries managers avoid spreading the parasite through contaminated fish stocks, and they may eventually point to ways to combat the parasite in the wild.

Although *M. cerebralis* poses no threat to humans who eat infected fish, whirling disease has jeopardized Montana's income from trout fishing, an industry that brings in $250 million a year. Anglers worldwide seek the thrill of catching Montana's wild rainbows, which leap, cartwheel, and run across the water. "Wild trout are part of our heritage," says Marshall Bloom, a medical virologist at the National Institutes of Health's Rocky Mountain Laboratories in Hamilton, Montana, and head of the Montana Whirling Disease Task Force.

When fish pathologist Beth MacConnell of the U.S. Fish and Wildlife Service in Bozeman first diagnosed whirling disease in the Madison River in late 1994, little was known about it. To this day, no one understands why whirling disease zeroes in on rainbows, how to stop it, or how it spread to Montana and 20 other states. But researchers hope that the new diagnostic technique, based on the polymerase chain reaction (PCR), may help clarify these critical questions.



Circle of death. The whirling disease parasite *M. cerebralis* passes from rainbow trout to mud to worms and back again into the trout.

Currently, whirling disease is diagnosed by a brute-force method: Fish heads are ground up and examined microscopically for spores of *M. cerebralis* that form as the disease progresses. Not only is this method time-consuming and inaccurate (related fish parasites produce similar-looking spores), it can only detect infections that have been developing for at least 4 months. Because a newly infected fish may look normal, says Ron Hedrick, director of the Fish Health Laboratory at the University of California, Davis, "they may have been stocked from hatcheries and spread the disease."

In 1996, Hedrick and his graduate student, Karl Andree—who in part were supported by the Whirling Disease Foundation—supplied the key ingredient needed for a PCR assay that can dodge many of these problems: They cloned and identified ribosomal RNA sequences specific to *M. cerebralis*. The assay works by finding bits of *M. cerebralis* DNA in tissue and then multiplying them up for detection. Early laboratory results by Andree confirm that the PCR assay can detect the parasite just 2 hours after infection.

Microbiologist Bob Ellis of Colorado State University in Fort Collins is now testing the assay in the field by placing young, caged fish in waters containing the parasite. After just 3 weeks of exposure, three of 29 fish tested positive in the tail area. After 2 months, nine of 39 fish tested positive in either their tails or heads. (*M. cerebralis* penetrates the tail epidermis first, then travels through nerve bundles to the head.) "The goal is to develop an early diagnostic method that doesn't require killing fish," says Ellis. When perfected, tail clippings of hatchery fish could be checked before dumping, preventing spread of the protozoa.

Screening hatchery fish will help in states that stock fish, but not in Montana: The state does not release hatchery fish in its streams—its reputation as a haven for fly fishers is built in part on the fact that its big trout are bred in the wild. So experts are looking for other ways to restore self-sustaining populations of wild rainbows in Montana and other states. That leads them to the mud-dwelling worm.

When fish die from whirling disease, *M. cerebralis* spores are released from decaying carcasses and survive for up to 30 years in mud. To infect fish, the spores must be eaten by mud-loving *Tubifex tubifex* worms. By some unknown mechanism, the worm's gut converts clam-shaped spores into an infectious form, *Triactinomyxon* (TAM), which look like three-armed hooks. "Without the worms, it's a dead end. Yet we know practically nothing about the worms," says Bloom.

Some fish biologists even doubted that the two morphologically distinct spore forms were related, especially as attempts to reproduce their complex life cycle in the laboratory brought inconsistent results. The PCR test put the controversy to rest, when Andree showed in the May-June *Journal of Eukaryotic Microbiology* that the spore and TAM stages share 99.8% genetic homology. And, for the first time, researchers can detect the parasite in *T. tubifex* worms. Before the PCR assay, "we had no diagnostic procedure for that at all," says Hedrick.

Of special interest are *T. tubifex* worms that appear to resist infection by the parasite. At the University of Montana, Missoula, parasitologist Bill Granath finds in preliminary studies that parasite DNA is detected in resistant worms 24 hours after spore infection, but is undetectable 4 days later. "It appears that parasite DNA enters the worm tissue, but doesn't develop," says Granath. These resistant worms fuel dreams about biological con-

LEFT: R. HEDRICK/UC DAVIS, RIGHT: B. MACCONNELL/USFWS

trol: If they could displace susceptible worms in the environment, they could offer a potential natural control. Hedrick's laboratory also plans to investigate how resistant worms neutralize spores and whether resistant genes can be passed to susceptible worms.

Granath's studies point to another potential weak spot in the parasite's life cycle: At 15 degrees Celsius, huge amounts of TAMs are released from the worms into the water, but at 5 degrees, few are released. This laboratory finding parallels field observations that hatchling rainbows, but not stream-sharing brown trout, contract whirling disease. Fish biologist Dick Vincent of Montana Fish, Wildlife, and Parks in Bozeman found that young rainbows emerge in May, when waters are warm and filled with TAMs that bombard them. Young brown trout emerge in March, when waters are colder and contain few TAMs. One implication is that selective pressures may eventually favor rainbow trout that spawn earlier in colder waters.

All these recent discoveries have resulted from a tiny research investment. In 1996, federal grants to study whirling disease totaled just $360,000, the Whirling Disease Foundation awarded $73,000, and some chapters of Trout Unlimited gave up to $10,000. Colorado State's Ellis says it may be time for anglers to "put more money into research instead of flies."

–Carol Potera

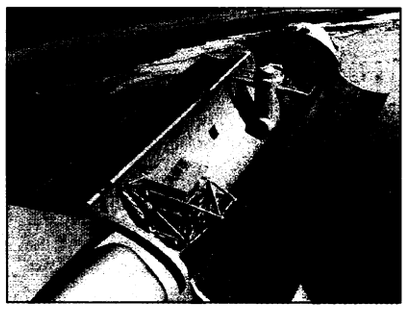
Carol Potera is a writer in Great Falls, Montana.

ASTROPHYSICS

Theorists Nix Distant Antimatter Galaxies

For those who yearn for an equal opportunity universe, half matter and half antimatter, the latest findings will be a disappointment: Matter dominates, and there are no antigalaxies, despite the dreams of science fiction fans everywhere. This is the conclusion of a trio of theorists after a lengthy analysis of the physics of matter-antimatter annihilation and the gamma-ray glow that pervades the sky. Their finding, to be reported in the *Astrophysical Journal* in February, may sound like a victory for conventional wisdom, but it underscores a long-standing mystery: why the big bang displayed such blatant favoritism toward matter.

The universe that sprang from the big bang should have contained equal parts of matter and antimatter. But cosmologists have long known that our cosmic neighborhood is all matter. Now physicists Andy Cohen of Boston University; Alvaro de Rújula of CERN, the European particle physics lab near Geneva; and Sheldon Glashow of Harvard University have confirmed that matter somehow dominated the rest of the visible universe as well. By rigorously calculating the energy that would have been emitted when matter and antimatter met and annihilated, then comparing the results with actual measurements of the gamma-ray background, they rule out the existence of large domains of antimatter. "It's probably the best job ever done of calculating the annihilation rates and the gamma-ray spectrum," says the University of Chicago's David Schramm.



Long shot? Artist's conception of the antimatter detector to fly on the space shuttle.

The favored explanation for the absence of antimatter in the nearby universe is that soon after the big bang a slight asymmetry developed between matter and antimatter. The asymmetry enabled a little extra matter to survive when the two annihilated, leaving a universe apparently devoid of antimatter. But that picture has been taken on faith more than data. The asymmetries in the relevant parameters of quantum physics—in particular, a phenomenon called CP violation—currently appear to be smaller than necessary.

The uncertainty opened the way for a second scenario: that the universe started off with equal amounts of matter and antimatter, segregated in nonoverlapping domains. When the newborn universe went through a spurt of exponential growth, called inflation, these domains grew so quickly that they never had time to annihilate completely. If so, the universe today would have huge domains of antimatter, on the scale of galaxy clusters or larger. These antigalaxies would look to us like the ordinary variety, but, says Cohen, there should be telltale signs of matter-antimatter annihilation at the boundaries between domains.

If the matter and antimatter domains are nearby in time and space, the high-energy gamma-rays from their boundaries would have been seen already, he says. But the signal from larger domains—at least the size of superclusters—could have been missed. Annihilation would have begun at their boundaries early in the history of the universe, says Cohen. "The gamma-rays would be smeared out, redshifted to lower energies by the expansion of the universe. Now, there is a diffuse gamma-ray background, and no one is exactly sure where it comes from. The suggestion that it comes from antimatter is an old one, going back several decades."

So he, de Rújula, and Glashow tested that idea by computing the spectrum of diffuse photons from matter-antimatter annihilation in the early universe. The process can be thought of as "the ultimate bomb," de Rújula says. A gas touching an antigas annihilates in an explosion of light, particles, and antiparticles, which in turn heats both the gas and antigas, causing them to annihilate faster, producing yet more annihilation and more heat and so on. ... While calculating the energy from this chain reaction is "pretty difficult," says de Rújula, "it's just a case of laboriously applying our knowledge to a very complicated thermodynamic calculation."

The three physicists conclude that even in the most conservative analysis, matter-antimatter annihilation should produce a signal five times as large as the observable diffuse gamma-ray background. "It's an awfully big effect," says Glashow.

Cohen notes that there are still a few loopholes: For instance, if the universe consists of just two domains, one entirely matter and the other entirely antimatter, the analysis wouldn't hold. "If you looked in one direction, you might not see any gamma-rays at all," he says. "So we don't have anything to say about that case."

Schramm says the analysis definitely reinforces the "prior prejudices" of theorists that the antimatter isn't there. But the work wasn't done just for the enlightenment of theorists. In 1995, physicist Sam Ting of CERN and the Massachusetts Institute of Technology began work on a detector to fly on the space station that would search for antimatter cosmic rays, such as nuclei of anticarbon, coming from distant antigalaxies (*Science*, 12 January 1996, p. 142). Ting's experiment is scheduled to be tested on the space shuttle this May.

Ting says he promised to buy de Rújula, Glashow, and Cohen dinner if their analysis supported the possibility that his detector will see cosmic antinuclei. Now dinner is off, he says. But Ting, who is famous for knowing when to ignore the predictions of theorists and won a Nobel Prize by doing just that, isn't discouraged. "The most important thing is that no precision experiment has ever been done" to measure the composition of cosmic rays. Adds Glashow, "We're not exactly saying it's impossible for [Ting] to find antimatter. We're saying that from the context of current cosmology it's impossible. So if he finds it, he upsets the whole apple cart."

–Gary Taubes